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Ethylene Suppression Modifies Gene Expression and Activity of Aroma Volatile-Related Enzymes in 'Delbard Estivale' Apples

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Abstract

The aim of this study was to assess the effect of AVG- and 1-MCP treatment on the postharvest behaviour of 'Delbarde Estivale' apple kept at room temperature, with special emphasis on the biosynthesis of odor volatiles. Fruits were harvested at the proper time, and subjected to AVG (ReTain®) treatment 3 weeks before harvest date. Another lot of fruits were treated with 1-MCP. Both treatments had a negative impact on the biosynthesis of odour-volatiles, in particular on ester-type compounds. The activities of lipoxygenase (LOX), alcohol dehydrogenase (ADH), and pyruvate decarboxylase (PDC) were declined by these treatments, whereas the alcohol o-acyltransferase (AAT) activity was higher in 1-MCP treated fruit. Gene expression assessment confirmed these trends. Results confirm the important role of an adequate supply of substrates for the biosynthesis of volatile esters in apple fruit.

INTRODUCTION

Aroma is a very important attribute for sensory quality of apple (*Malus domestica* Borkh.) fruit. In spite of this relevance, post-harvest procedures have focused preferentially on other quality aspects such as firmness and visual quality, and aroma has been generally disregarded. Apple fruit produce many aroma-related volatile compounds, but the most important contributors to fruit flavour are esters. Ethylene is required for the biosynthesis of volatile esters in apple, as it is known to modulate the expression of alcohol o-acyltransferase (AAT) (Defilippi et al., 2005), which catalyses the last step in the biochemical pathway, and is thus directly responsible for the generation of this kind of compounds. The relationship between the biosynthesis of odour volatiles and ethylene is important, since the usage of ethylene suppression chemicals, mainly AVG and 1-MCP, is increasing. Mir et al. (1999) found that AVG inhibited volatile production by 'Jonagored Jonagold' apple fruit, which appeared to be independent of respiration. Also, Fan et al. (1998) found that the synthesis of certain volatiles was inhibited by AVG treatment of preclimacteric fruits. Concerning 1-MCP, Li et al. (2006) stated that although 1-MCP treatment prolonged post-harvest life of apples, it repressed the regeneration of esters during post-storage ripening.

'Delbard Estivale' is an early apple variety with sweet flavour, although a bit more acid than that of 'Golden Delicious'. The aim of this study was to assess the effect of AVG- and 1-MCP treatment on the generation of volatile compounds during the post-harvest life of 'Delbard Estivale' apples kept at room temperature, and to determine which steps in the biochemical pathway are dependent on ethylene regulation.

MATERIALS AND METHODS

Plant Material and Ethylene Suppression Treatments

'Delbard Estivale' apples were harvested at commercial maturity and selected for uniform fruit size and appearance. AVG (ReTain®) was applied 3 weeks before harvest (125 ppm a.i.). Upon harvest, half of the control fruit were treated with 1-MCP (0.625 ppm for 24h at 10°C). Samples were kept subsequently at 20°C for up to 3 weeks, and analyzed periodically as indicated below.

Aroma Volatiles

Volatile compounds were extracted and analyzed as in Harb et al. (2007).

Gene Expression

Total RNA was isolated as described in Chang et al. (1993). 20 µg of total RNA were electrophoresed in denaturing buffer, blotted to a Hybond-N^+ nylon membrane, and fixed according to standard procedures. Blots were hybridized with [α-^{32}P]dCTP-labelled DNA probes.

Aroma-Related Enzyme Activities

LOX, PDC, ADH and AAT activities were extracted and assayed on crude enzyme extracts obtained from unpeeled fruit cortex as described elsewhere (Lara et al., 2003). Total protein content in the enzyme extract was determined with the Bradford (1976) method, using BSA as the standard.

Statistical Analysis

Data were subjected to analysis of variance, and mean separations were calculated by Duncan's Multiple Range Test at $P \leq 0.05$.

RESULTS

Respiration and Ethylene Production Rates

Both 1-MCP and AVG treatments significantly delayed ripening, as shown by significantly inhibited respiration and ethylene rates as compared to untreated samples, with no significant differences between both ethylene suppression treatments (data not shown).

Volatile-Related Enzyme Activities

LOX enzyme was inhibited in response to both ethylene suppression treatments (Table 1). This suppression of LOX activity through these treatments remained even after three weeks, although a slight recovery could be observed for 1-MCP-treated fruits. PDC and ADH activities were significantly decreased by both ethylene suppression treatments assessed. In contrast, AAT activity was enhanced in 1-MCP-treated samples, whereas no significant differences as compared to untreated controls were observed for AVG-treated apples.

Gene Expression Analysis

This analysis revealed that ADH gene expression was significantly higher for control fruit as compared to both 1-MCP- and AVG-treated samples (Fig. 1). However, both ethylene suppression treatments, and particularly 1-MCP, enhanced gene expression of AAT, consistent with observations for the corresponding enzyme activity levels as seen in Table 1.

Odour-Volatile Profiles

Both ethylene suppression treatments had a negative impact on the total emission of volatile compounds (Table 2). When considering specifically the production of volatile

esters, important changes were also found in response to both treatments. However, not all the esters were equally affected. For branched-chain esters, no consistent trend could be observed, suggestive of the coexistence of both ethylene-dependent and independent production patterns for this family of volatile esters. In contrast, the biosynthesis of most straight chain esters was drastically reduced upon treatment with 1-MCP and AVG (Table 2), although these effects were more intense for 1-MCP-treated fruit. Butyl and hexyl esters, particularly prominent in the volatile blend emitted by fruit, were especially affected. As to alcohols and aldehydes, the emission of 1-butanol and 1-hexanol (data not shown) was clearly reduced by both AVG and 1-MCP treatments, in accordance with the decrease in butyl and hexyl esters.

DISCUSSION

Aroma remains an important quality attribute, and certain new technologies (e.g., the usage of 1-MCP) reportedly have a negative impact thereupon. Taking into account the important role that ethylene plays in the biosynthesis of odour volatiles (Bangerth, 1984; Defilippi et al., 2005), the impact of 1-MCP and AVG treatments is obvious. Concerning the enzymatic machinery of odour-volatiles biosynthesis, AAT is the direct enzyme responsible for ester synthesis and thus a key step in this process (Fellman et al., 2000; Beekwilder et al., 2004), which is believed to be ethylene-dependent (Defilippi et al., 2005). On the other hand, fruit ripening includes also ethylene-independent processes (Silverman et al., 2004). Since the biosynthesis of volatile esters in fruit is also limited by the concentration of the necessary alcohol substrates (Berger and Drawert, 1984), a significant role in volatile ester production has been suggested for enzymes providing these or other precursors, such as ADH or LOX (Echeverría et al., 2004). Specifically, the availability of alcohols is believed to be a bottle-neck for ester production (Beekwilder et al., 2004), with the last step of ester formation being driven by AAT, which catalyzes a transacylation from an acyl-CoA to an alcohol acceptor.

The lack of precursors has been shown to be a serious limiting factor for volatile production, while the enzymatic machinery needed for ester formation seems less restrictive (Harb et al., 2000). This is consistent with results reported herein, showing decreased production of volatile esters in spite of similar (AVG) or even increased (1-MCP) AAT activity levels in treated fruit (Table 1). *AAT* gene expression levels were also higher for treated fruit (Fig. 1), and therefore it was obvious that the modifications in AAT activity or gene expression were not in agreement with the decrease in ester production found for treated fruit (Table 2). Hence, decreased ester production must have arisen from restrictions in substrate availability, and indeed alcohol emission was severely depleted in treated fruit. Inhibited emission of alcohols arose at least partially from decreased activity (Table 1) and gene expression (Fig. 1) levels for ADH, which provides alcohols for subsequent AAT action.

Other aroma-related enzyme activities were also dependent on ethylene levels. For instance, PDC activity, which provides aldehydes for ADH-catalyzed reduction to alcohols, was significantly inhibited in treated fruit. Similarly, significantly and lastingly decreased levels of LOX activity for both ethylene suppression treatments (Table 1) suggested that this aroma-related enzyme activity was also ethylene-related. The key role of LOX in aroma volatile biosynthesis is further strengthened by the observation that HPL activity was promoted initially by 1-MCP treatment (data not shown), suggesting that this activity, responsible for the cleavage of fatty acid hydroperoxides to aldehydes and oxoacids (Vancanneyt et al., 2001), was not a limiting factor for aroma volatile-synthesizing capacity.

Provided the necessary precursors are not limiting, pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), and alcohol o-acyltransferase (AAT) determine ultimately the final composition of volatile profile emitted by fruits. PDC and ADH activity levels were significantly inhibited by both ethylene suppression treatments, suggesting ethylene is required for the expression or activity of the corresponding gene product. Contrarily, AAT activity was enhanced in 1-MCP-treated fruit, but in contrast it was apparently

unaffected by AVG applications. PDC and ADH activities have been shown to be increased by some CA treatments (Ke et al., 1993), whereas AAT activity has been reported to be suppressed upon the exposure of apple fruit to 0.5 or 1 kPa O_2 (Ke et al., 1994), under which conditions ethylene production and action are usually limited.

In conclusion, the impaired biosynthesis of aroma volatiles by 'Delbard Estivale' apples in response to AVG and 1-MCP appeared to be related to early events in the chain that supply precursors for the enzymatic machinery that deliver volatiles, particularly to ADH, PDC and, to a lesser extent, LOX activities. However, impairments resulted from lower respiration rate cannot be excluded.

ACKNOWLEDGEMENTS

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Tables

Table 1. Influence of AVG and 1-MCP treatments on different aroma volatile-related enzyme activities (U mg protein^{-1}) extracted from 'Delbard Estivale' apple fruits kept at room temperature after harvest[a].

	Control	AVG	MCP
		LOX	
1 week	8.46a	3.85b	10.01a
2 weeks	11.34a	7.75b	9.44ab
3 weeks	15.17a	11.01b	12.56b
		PDC	
1 week	14.6a	7.29c	7.97b
2 weeks	24.61a	5.97c	7.94b
3 weeks	19.07a	5.51c	7.63b
		ADH	
1 week	17.21a	8.29b	8.95b
2 weeks	15.46a	9.12c	13.59b
3 weeks	14.17a	7.74b	9.10b
		AAT	
1 week	0.014b	0.016ab	0.018a
2 weeks	0.015b	0.014b	0.021a
3 weeks	0.015a	0.017a	0.017a

[a] Values are the means of three replicates. Different letters within the same row indicate significant differences at $p \leq 0.05$.

Table 2. The influence of AVG and 1-MCP treatments on the biosynthesis of selected odour volatile families (μg/kg FW^{-1})[a].

	Control			AVG			MCP		
	7	14	21	7	14	21	7	14	21
BC esters	1.45	1.56	1.29	0.73	1.31	1.35	1.04	1.05	0.67
SC esters	4.46	5.22	3.90	2.60	4.64	3.32	1.58	2.00	2.47
Butyl esters	1.76	2.06	1.85	0.47	1.01	1.44	0.47	0.52	0.86
Hexyl esters	3.43	3.47	2.46	2.37	3.88	2.13	1.42	1.77	1.72
Total esters	5.91	6.78	5.19	3.33	5.94	4.67	2.62	3.05	3.13
Alcohols	1.91	2.17	3.23	0.67	1.79	2.03	0.92	0.91	0.65
Aldehydes	1.94	2.89	1.96	1.76	2.57	1.75	2.46	1.65	1.85
Other	1.54	3.97	2.18	0.10	0.44	1.76	0.30	0.73	1.10
Total volatiles	11.31	15.80	12.56	5.86	10.74	10.21	6.30	6.35	6.74

[a] Values are the means of three replicates (BC esters, branched-chain esters; SC esters, straight-chain esters).

Figures

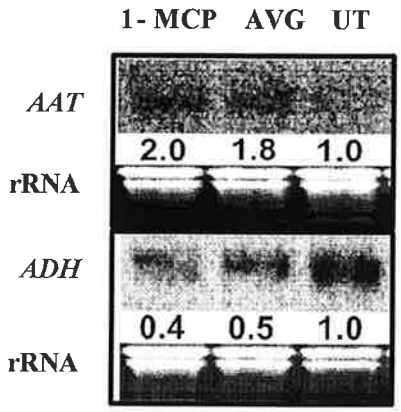

Fig. 1. RNA gel blots for untreated, AVG- and 1-MCP-treated 'Delbard Estivale' apple fruit hybridized with *ADH* and *AAT* probes. Samples were taken seven days after treatments. The hybridization signals were normalized to the rRNA bands. Numbers indicate the relative *ADH* and *AAT* mRNA levels (UT, untreated).

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